2013. The Journal of Arachnology 41:31–35

Facilitation of ground-dwelling wolf spider predation on mirid bugs by horizontal webs built by *Tetragnatha* spiders in organic paddy fields

Mayura B. Takada[1], **Tetsuya Kobayashi**[2], **Akira Yoshioka**[3], **Shun Takagi**[3] and **Izumi Washitani**[3]: [1]Laboratory of Animal Ecology, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan. E-mail: mayura@obihiro.ac.jp; [2]National Institute of Agrobiological Sciences, Owashi Tsukuba, Ibaraki 51, 305-0851 Japan; [3]Graduate School of Agricultural and Life Sciences, University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

Abstract. Trait-mediated effects of predators can impact prey population dynamics by affecting prey behavior. The mirid bug *Stenotus rubrovittatus* (Matsumura) (Hemiptera: Miridae), a major insect pest in Japanese rice production, usually remains in the upper layer of paddies to feed on rice ears. However, the mirids are frequently trapped by horizontal webs of *Tetragnatha* spp. spiders, which are highly abundant in organic rice paddies, and fall to the bottom layers of paddies where they are preyed upon by ground-dwelling predators. It is hypothesized that *Tetragnatha* spp. spiders facilitate bug predation by wolf spiders through trait-mediated effects, in which their horizontal webs force the bugs onto or near the ground and thereby into the hunting zones of wolf spiders. Molecular gut-content analysis of 619 wolf spiders coupled with field measurements revealed that the number of wolf spiders that tested positive for mirid bug predation increased significantly with the density of *Tetragnatha* spp. spiders in the paddies. We also observed a positive relationship between *Tetragnatha* spp. abundance and total cover by their webs in paddies. We identified the potential for an unexpected interaction between an herbivorous insect pest and ground-dwelling spiders that usually inhabit different microhabitats in paddy fields by focusing on trait-mediated effects of webs built by *Tetragnatha* spp. Because spider webs occupy a certain proportion of the available space in terrestrial ecosystems, consideration of trait-mediated effects on interactions between flying insects and other predators may lead to a better understanding of local food webs.

Keywords: Ecosystem function, molecular-gut content analysis, natural enemy, pest management, trait-mediated effect

Predators affect prey populations through both density-mediated and trait-mediated effects, which can extend throughout the food web (Werner & Peacora 2003; Schmitz 2010). Trait-mediated effects are mainly mediated by behavioral changes in prey or other organisms, including reductions in feeding time (Griffin & Thaler 2006) and emigration from a particular microhabitat (Nakasuji et al. 1973; Losey & Denno 1998). These effects can cause indirect changes in the biomass, diversity (Schmitz 2010) or quality of food that is available to predators (Griffin & Thaler 2006) in both natural and agricultural ecosystems.

The webs woven by spiders might not only function as passive traps that catch prey but could also interfere with insect flight behaviors by forcing individuals to avoid spider webs (Rypstra 1982; Craig 1986; Blackledge & Wenzel 1998) or through attraction by web silk decorations (Craig & Bernard 1990; Tso 1998; Watanabe 1999). These trait-mediated effects of webs may significantly alter the biological interactions between flying insects and other predators, but few studies have explicitly tested this possibility.

Spiders are ubiquitous predators in rice paddies. In northern Japanese organic paddies that are managed using few or no chemical applications, *Tetragnatha* spp. (Araneae: Tetragnathidae), horizontal web weavers that live in the canopies of rice paddies, are conspicuously abundant (Oyama et al. 2005; Amano et al. 2011). The most serious threat to rice production in this area is grain discoloration caused by *Stenotus rubrovittatus* (Matsumura) (Hemiptera: Miridae). They prey on ears of wild poaceous plants and cultivated rice. From source populations in meadows and fallows (Yoshioka et al. 2011), adult bugs spill over into rice paddies after rice plants start heading and infest rice grains (Takada et al. 2012). Our observational study (Takada et al. 2012) indicated that *Tetragnatha* spp. decrease the abundance of the mirid bug *S. rubrovittatus* and reduce the amount of damage caused by the mirid, suggesting that the spiders act as a natural enemy against the bugs.

In our study paddies, *S. rubrovittatus* is frequently observed falling to the bottom layer of the paddies after becoming tentatively trapped by horizontal webs of *Tetragnatha* spp. in the rice canopy. The webs seem to be too fragile to catch the bugs and are better adapted for catching small flies, such as chironomids (Kato et al. 2003), which are considerably smaller than the mirids. Bugs that fall to the bottom layer are exposed to ground-dwelling predators such as wolf spiders (Lycosidae), which hunt on the ground or in the bottom layer (e.g., Kiritani et al. 1970), and are likely to be preyed upon by them. The mirid bugs usually stay and feed on ears of rice in the uppermost vegetation layer (Takada et al. 2012).

This study was conducted to test the hypothesis that horizontal webs woven by *Tetragnatha* spp. enhance *S. rubrovittatus* predation by wolf spiders, which are abundant predators in the bottom layer. We analyzed relationships between mirid bug predation and measured densities of *Tetragnatha* spp. spiders, total coverage of horizontal webs, and the number of wolf spiders. Molecular gut-content analysis using DNA markers specific for *S. rubrovittatus* (Sheppard & Harwood 2005; King et al. 2008; Kobayashi et al. 2011) facilitated our evaluation of the magnitude of wolf spider predation.

METHODS

Study sites.—The study was conducted in Osaki City, Miyagi Prefecture, Northern Japan (38°37′N, 141°07′E) in August 2008. Annual precipitation in the area was 1126 mm, and mean temperature in August 2008 was 22.5°C (Japan Meteorological Agency 2010). In this area, community-based, biodiversity-friendly farming activities have been expanding recently (Kurechi 2007).

Field survey.—Nineteen paddy fields that had similar management without the application of chemical herbicides or insecticides were chosen within an area of about 20 km^2. Two spider groups, *Tetragnatha* spp. and wolf spiders, were the most abundant spider groups in the paddy fields chosen for this study (Oyama and Kidokoro 2003; Takada et al. 2012). A field survey was conducted in August 2008, when rice ears were fully emerged and the mirids were at peak density. The densities of *Tetragnatha* spp. and *S. rubrovittatus* were measured at the center of each paddy field using net sampling; 20 sweeps were performed with a 36-cm-diameter sweeping net. At the same time, chironomid abundance was also measured because chironomids are known to be an important alternative prey for wolf spiders in paddy fields (Settle et al. 1996; Ishijima et al. 2006), and we hypothesized that abundant alternative prey interfere with mirid predation by wolf spiders (Harwood et al. 2004; Kuusk & Ekbom 2010; Öberg et al. 2011). To estimate the availability of alternative prey for wolf spiders, body lengths of chironomids were measured to the nearest 0.1mm with a measuring ocular on a stereo microscope, and their body mass was calculated in each field using the equation mass = 0.00305 × (body length)$^{2.62}$ (Rogers et al. 1976). Body mass was used instead of density because body length of these prey varied widely. Wolf spider density was estimated by direct counting. We walked through the rice hills (sheaves consisting of several rice stems) in a straight line along a row of 15 rice hills per field. When wolf spiders were observed in and around hills, the individuals were counted.

It was difficult to measure web coverage in all of the 19 study fields because *Tetragnatha* spp. build webs mainly from dusk till dawn (Kiritani et al. 1972; Tahir et al. 2009). Therefore, we used *Tetragnatha* spp. density, measured during the net sampling, as an index of the coverage of their webs in each paddy field, after testing for a positive relationship between the densities of spiders and webs in eight study fields (see Statistical Analyses). The coverage of *Tetragnatha* spp. webs was measured in three quadrats (1 × 1 m) set in the center of each of eight study paddy fields just before dusk or just after dawn. To enhance the clarity of webs for observation, the webs were misted with water using an atomizer. The mean area of webs in the three quadrats was used as an index of the coverage of *Tetragnatha* spp. webs in each paddy field.

Prey detection evaluation.—At the center of each study site, approximately 30 wolf spiders larger than 4 mm in body length were collected. We assumed that spiders smaller than this critical size could not consume the bugs, which had adult body sizes of 4.22 ± 0.49 (mean ± SD) mm (n = 32). Collected spiders were transferred to vials containing 80% ethanol, identified to species as well as sex and developmental stage (adult or juvenile), and placed in a freezer at −20°C until laboratory gut-content analysis. We investigated whether each spider had recently fed on *S. rubrovittatus* by testing for the presence of bug DNA in their gut contents (Kobayashi et al. 2011). In short, DNA was extracted from the abdomen of the spiders. A 250 bp DNA fragment from the mitochondrial cytochrome c oxidase subunit I (*COI*) gene of the target prey was amplified by polymerase chain reaction (PCR) using *S. rubrovittatus* specific primers. Amplified DNA was verified by electrophoresis in agarose gel. The proportion of individuals that tested positive was estimated by dividing the number of individuals that tested positive for bug DNA by the total number of spiders analyzed in each study field.

Data for abundances of spiders, *S. rubrovittatus*, and chironomids, and the proportion of individuals that tested positive for bug DNA in wolf spiders were obtained from a dataset created during our previous study (Kobayashi et al. 2011). However, the goals and hypotheses of the present study are different from those of Kobayashi et al. (2011).

Statistical analyses.—To test whether horizontal web cover increased with the abundance of *Tetragnatha* spp., a simple linear regression analysis was performed using cover as a dependent variable and *Tetragnatha* spp. density as an independent variable. We then applied a multiple logistic regression analysis to test whether increases in the abundance of *Tetragnatha* spp. were associated with increases in the proportion of wolf spiders testing positive for *S. rubrovittatus* DNA; we used the abundances of *Tetragnatha* spp. spiders, wolf spiders, and the bugs, and chironomid biomass as independent variables. To check for collinearity between the independent variables, tolerance values were compared to the critical value of 0.1 (Quinn & Keough 2002). All statistical analyses were performed using R for Windows 2.13.1 (*R* Development Core Team 2011).

RESULTS

The dominant *Tetragnatha* species were *Tetragnatha caudicula* Karsch 1879, *Tetragnatha extensa* Linnaeus 1785, *Tetragnatha maxillosa* Thorell 1895 and *Tetragnatha praedonia* L. Koch 1878. A positive relationship was found between the density of *Tetragnatha* spp. and the area covered by their horizontal webs, although it was marginal ($F_{1,6}$ = 4.814, P = 0.071; Fig. 1). The highest web cover in the study fields was 25.8%.

All of the wolf spiders collected during the study period were *Pirata subpiraticus* Bösenberg & Strand 1906. In total, 691 *P. subpiraticus* individuals from 19 study fields were analyzed for gut content. A multiple logistic regression analysis showed that the proportion of individuals that tested positive the bug DNA was related positively to the density of *Tetragnatha* spp. and negatively to chironomid biomass (Table 1, Fig. 2). All tolerance values were greater than the critical value of 0.1, indicating that there was no significant collinearity between the independent variables (Table 1).

DISCUSSION

Molecular gut-content analysis revealed that mirid predation by wolf spiders increased with the density of *Tetragnatha* spp. in paddy fields. This partially supports our hypothesis that *Tetragnatha* spp. spiders facilitate bug predation by wolf spiders through trait-mediated effects. We collected wolf spiders for the gut-content analysis during the daytime, although feeding activity of these spiders seems to be more intensive from dusk till dawn (Kiritani et al. 1972), as does that of the bugs (Butler 1972; Mueller & Stern 1973).

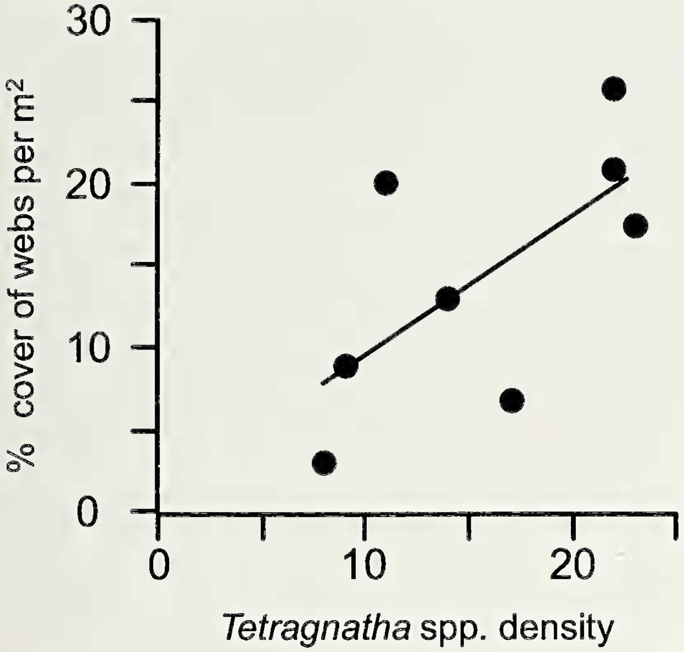

Figure 1.—Relationship between the density of *Tetragnatha* spp. spiders and the area covered by their webs per m^2. Estimated linear regression line ($y = 0.850x + 1.043$; $r^2 = 0.445$) is shown.

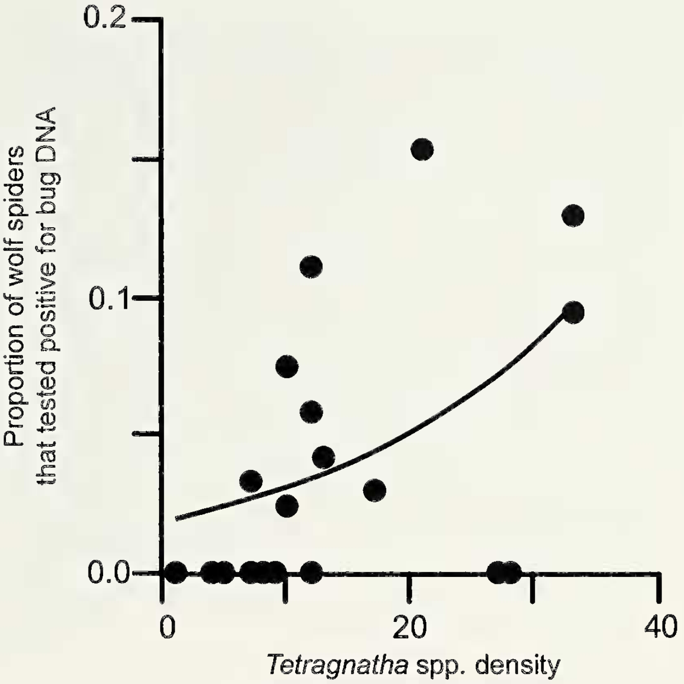

Figure 2.—Relationship between the density of *Tetragnatha* spp. spiders and the proportion of individuals tested positive for bug DNA in wolf spiders. Estimated logistic regression line is shown.

However, the sampling is unlikely to underestimate the spider predation on the bugs because the detection half-life (e. g., Chen et al. 2000) of *S. rubrovittatus* DNA in wolf spiders' guts was long (approximately 3.4 days at 25°C; Kobayashi et al. 2011). Our observation that their horizontal webs force the bugs to relocate onto or near the ground, into the hunting zone of wolf spiders (M.B. Takada pers. observ.) would be the primary cause of this trait-mediated effect. The positive relationship between *Tetragnatha* spp. density and the coverage of their webs strengthens this inference, although it would be more accurate to take *Tetragnatha* spp. web coverage measures of all of the 19 study fields and test the relationship between mirid bug predation by wolf spiders and the web coverage directly. In addition, in a future study, we will test whether the horizontal webs of *Tetragnatha* spp. cause bugs to drop to the surface of the water, and whether their effects influence bug density and bug-induced crop damage in paddy fields.

Chironomid biomass decreased mirid predation by wolf spiders. It is known that dipterans such as chironomids are important alternative prey for spiders in paddies (Ishijima et al. 2006; Tahir & Butt 2009). Abundant alternative prey may interfere with mirid predation by wolf spiders. Previous studies have also revealed negative relationships between insect pest consumption by spiders and availability of alternative prey in crop fields (Harwood et al. 2004; Kuusk & Ekbom 2010; Öberg et al. 2011).

The top-down effect of *Tetragnatha* spp. spiders on the bugs and the amount of crop damage that was demonstrated in our observational study (Takada et al. 2012) can be attributed to the trait-mediated effect of the horizontal webs of *Tetragnatha* spp. In the bottom layer in paddy fields, there are many generalist predators besides wolf spiders that are larger than the bugs, including other hunting spiders, such as *Pachygnatha clercki* Sundevall 1823 (Oyama et al. 2005; Takada et al. 2012), and water striders, such as *Gerris* spp. (M.B. Takada, pers. observ.). These predators might also prey upon bugs when they fall to the ground after being trapped by *Tetragnatha* spp. webs. Predation by ground-dwelling predators on aphids that fall from plants has been reported in alfalfa fields (Losey & Denno 1998).

The enhancement of wolf spider predation on the mirid bugs by *Tetragnatha* spp. spider webs can be classified as a synergistic or substitutable effect (Schmitz 2007) between two generalist predators on the bugs. Although previous studies stressed that enhancement of biological control functions by increasing diversity of generalist predators is unlikely or

Table 1.—Multiple logistic regression results and tolerance values between independent variables.

Variables	Tolerance values	Estimate	SE	χ^2	*P*
Tetragnatha spp. density	0.466	0.079	0.025	9.843	0.002
Wolf spider density	0.936	0.012	0.018	0.478	0.489
Chironomid biomass	0.614	−0.021	0.010	8.897	0.003
Bug density	0.738	0.016	0.009	3.757	0.053

limited due to antagonistic interactions, such as intra-guild predation (e.g., Finke & Denno 2004; Denno et al. 2004), we have demonstrated a circumstance in which two spider groups do not spatially share habitat domains (Schmitz 2010). In our system, *Tetragnatha* spp. inhabit webs in the uppermost layer, whereas wolf spiders hunt on the ground or in the bottom paddy layer.

We found the potential for an unexpected interaction between an herbivorous insect pest and ground-dwelling spiders that usually inhabit different microhabitats in paddy fields by focusing on trait-mediated effects of webs built by *Tetragnatha* spp. Since *Tetragnatha* spp. spiders are also dominant in riparian ecosystems (Henschel et al. 2001; Kato et al. 2003; Iwata 2007) and usually build horizontal webs at the water surface, the trait-mediated effects of their webs might affect relationships between terrestrial and aquatic food webs (Nakano & Murakami 2001) by subsidizing terrestrial prey to aquatic predators. As spiders are ubiquitous predators in terrestrial ecosystems (Wise 1993), their webs should occupy a certain proportion of the available space. Therefore, consideration of the trait-mediated effects of spider webs on interactions between flying insects and other predators may lead to a better understanding of local food webs.

ACKNOWLEDGMENTS

This study was made possible through the help of organic rice farmers in Osaki City and by Osaki City Hall. We also thank J. Oyama, S. Iwabuchi, T. Miyashita, T. Kubo, and Y.G. Baba for providing helpful comments, A. Tanikawa for identification of spiders, and M. Sasaki for technical assistance. This study was made possible by a Grant-in-Aid for Young Scientists (B) (23780049) and the 21st Century COE Program at the University of Tokyo "Biodiversity and Ecosystem Restoration Research Project."

LITERATURE CITED

Amano, T., Y. Kusumoto, H. Okamura, Y.G. Baba, K. Hamasaki, K. Tanaka & S. Yamamoto. 2011. A macro-scale perspective on within-farm management: how climate and topography alter the effect of farming practices. Ecology Letters 14:1263–1272.

Blackledge, T.A. & J.W. Wenzel. 1998. Do stabilimenta in orb webs attract prey or defend spiders? Behavioral Ecology 10:382–376.

Butler, G.D. Jr. 1972. Flight times of *Lygus hesperus*. Journal of Economic Entomology 65:1299–1300.

Chen, Y., K.L. Giles, M.E. Payton & M.H. Greenstone. 2000. Identifying key cereal aphid predators by molecular gut analysis. Molecular Ecology 9:1887–1898.

Craig, C.L. 1986. Orb-web visibility: the influence of insect flight behaviour and visual physiology on the evolution of web designs within the Araneoidea. Animal Behaviour 34:54–68.

Craig, C.L. & G.D. Bernard. 1990. Insect attraction to ultraviolet-reflecting spider webs and web decorations. Ecology 71:616–623.

Denno, R.F., M.S. Mitter, G.A. Langellotto, C. Gratton & D.L. Finke. 2004. Interactions between a hunting spider and a web-builder: consequences of intraguild predation and cannibalism for prey suppression. Ecological Entomology 29:566–577.

Finke, D.L. & R.F. Denno. 2004. Predator diversity dampens trophic cascades. Nature 429:407–410.

Griffin, C.A. & J.S. Thaler. 2006. Insect predators affect plant resistance via density- and trait-mediated indirect interactions. Ecology Letters 9:335–343.

Harwood, J.D., K.D. Sunderland & W.O.C. Symondson. 2004. Prey selection by linyphiid spiders: molecular tracking of the effects of alternative prey on rates of aphid consumption in the field. Molecular Ecology 13:3549–3560.

Henschel, J.R., D. Mahsberg & H. Stumpf. 2001. Allochthonous aquatic insects increase predation and decrease herbivory in river shore food webs. Oikos 93:429–438.

Ishijima, C., A. Taguchi, M. Takagi, T. Motobayashi, M. Nakai & Y. Kunimi. 2006. Observational evidence that the diet of wolf spiders (Araneae: Lycosidae) in paddies temporarily depends on dipterous insects. Applied Entomology and Zoology 41:195–200.

Iwata, T. 2007. Linking stream habitats and spider distribution: spatial variations in trophic transfer across a forest–stream boundary. Ecological Research 22:619–628.

Japan Meteorological Agency. 2010. Weather, Climate & Earthquake Information. Online at http://www.jma.go.jp/jma/indexe.html

Kato, C., T. Iwata, S. Nakano & D. Kishi. 2003. Dynamics of aquatic insect flux affects distribution of riparian web-building spiders. Oikos 103:113–120.

King, R.A., D.S. Read, M. Traugott & W.O.C. Symondson. 2008. Molecular analysis of predation: a review of best practice for DNA-based approaches. Molecular Ecology 17:947–963.

Kiritani, K., N. Hokyo, T. Sasaba & F. Nakasuji. 1970. Studies on population dynamics of the green rice leafhopper, *Nephotettix cincticeps* UHLER: Regulatory mechanism of the population density. Researches on Population Ecology 12:137–153.

Kiritani, K., S. Kawahara, T. Sasaba & F. Nakasuji. 1972. Quantitative evaluation of predation by spiders on the green rice leafhopper, *Nephotettix cincticeps* Uhler, by a sight-count method. Researches on Population Ecology 13:187–200.

Kobayashi, T., M. Takada, S. Takagi, A. Yoshioka & I. Washitani. 2011. Spider predation on a mirid pest in Japanese rice fields. Basic and Applied Ecology 12:532–539.

Kurechi, M. 2007. Restoring rice paddy wetland environments and the local sustainable society—Project for achieving coexistence of rice paddy agriculture with waterbirds at Kabukuri-numa, Miyagi prefecture, Japan. Global Environmental Research 12:141–152.

Kuusk, A. & B. Ekbom. 2010. Lycosid spiders and alternative food: feeding behavior and implications for biological control. Biological Control 55:20–26.

Losey, J.E. & R.F. Denno. 1998. The escape response of pea aphids to foliar-foraging predators: factors affecting dropping behaviour. Ecological Entomology 23:53–61.

Mueller, A.J. & V.M. Stern. 1973. *Lygus* flight and dispersal behavior. Environmental Entomology 2:361–364.

Nakano, S. & M. Murakami. 2001. Reciprocal subsidies: dynamic interdependence between terrestrial and aquatic food webs. Proceedings of the National Academy of Science of the USA 98:166–170.

Nakasuji, F., H. Yamanaka & K. Kiritani. 1973. The disturbance effect of micryphantid spiders on the larval aggregation of the tobacco cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae). Kontyu 41:220–227.

Öberg, S., A. Cassel-Lundhagen & B. Ekbom. 2011. Pollen beetles are consumed by ground- and foliage dwelling spiders in winter oilseed rape. Entomologica Experimentalis et Applicata 138:256–262.

Oyama, J. & T. Kidokoro. 2003. Effect of predation by spiders on density of the green rice leafhopper, *Nephotettix cincticeps*, in cool area of Japan. Annual Report of Society of Plant Protection in North Japan 54:126–129. [In Japanese]

Oyama, J., T. Kidokoro & T. Ono. 2005. Effect of pesticide on natural enemy in paddy field. Bulletin of the Miyagi Prefectural Furukawa Agricultural Experiment Station 5:31–42. [In Japanese with English summary.]

Quinn, G.P. & M.J. Keough. 2002. Experimental design and data analysis for biologists. Cambridge University Press, Cambridge, UK.

R Development Core Team. 2011. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Online at http://www.R-progect.org

Rogers, L.E., W.T. Hinds & R.L. Buschbom. 1976. A general weight vs. length relationship for insects. Annals of the Entomological Society of America 69:387–389.

Rypstra, A.L. 1982. Building a better insect trap. An experimental investigation of prey capture in a variety of spider webs. Oecologia 52:31–36.

Schmitz, O.J. 2007. Predator diversity and trophic interactions. Ecology 88:2415–2426.

Schmitz, O.J. 2010. Resolving ecosystem complexity. Princeton University Press, Princeton, New Jersey.

Settle, W.H., H. Ariawan, E.T. Astuti, W. Cahyana, A.L. Hakin, D. Hindanaya, A.S. Lestari & P. Sartanto. 1996. Managing tropical rice pests through conservation of generalist natural enemies and alternative prey. Ecology 77:1975–1988.

Sheppard, S.K. & J.D. Harwood. 2005. Advances in molecular ecology: tracking trophic links through predator-prey food-webs. Functional Ecology 19:751–762.

Takada, M.B., A. Yoshioka, S. Takagi, S. Iwabuchi & I. Washitani. 2012. Multiple spatial scale factors affecting mirid bug abundance and damage level in organic rice paddies. Biological Control 60:80–85.

Tahir, H.M. & A. Butt. 2009. Predatory potential of three hunting spiders inhabiting the rice ecosystems. Journal of Pest Science 82:217–225.

Tahir, H.M., B. Abida & S.S. Muhammad. 2009. Foraging strategies and diet composition of two orb web spiders in rice ecosystems. Journal of Arachnology 37:357–362.

Tso, I.-M. 1998. Stabilimentum-decorated webs spun by *Cyclosa conica* (Araneae, Araneidae) trapped more insects than undecorated webs. Journal of Arachnology 26:101–105.

Watanabe, T. 1999. Prey attraction as a possible function of the silk decoration of the uloborid spider *Octonoba symotides*. Behavioral Ecology 10:607–611.

Werner, E.E. & S.D. Peacora. 2003. Review of trait-mediated indirect interactions in ecological communities. Ecology 84:1083–1100.

Wise, D.H. 1993. Spiders in ecological webs. Cambridge University Press, New York.

Yoshioka, A., M. Takada & I. Washitani. 2011. Facilitation of a native pest of rice, *Stenotus rubrovittatus* (Hemiptera: Miridae), by the non-native *Lolium multiflorum* (Cyperales: Poaceae) in an agricultural landscape. Environmental Entomology 40:1027–1035.

Manuscript received 9 May 2012, revised 22 October 2012.